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## REMARKS

Claims 51-93 are pending in the instant application. Reconsideration of this application in view of the amendments presented above and the remarks presented below is respectfully requested.

### Information Disclosure Statement

The Examiner indicates that reference C10 of the Information Disclosure Statement filed July 14, 2003, has not been reviewed. Applicants have included a copy of reference C10 herewith, and respectfully request review of the reference.

### 35 USC § 112, second paragraph

Claims 71 and 72 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. In particular, the Examiner asserts that Claim 71 is indefinite due to its dependency from Claim 70. In addition, the Examiner asserts that Claim 72 lacks antecedent basis in its recital of “n is  $\geq 6$ .” In light of the amendments, presented above, Applicants submit that the claims are sufficiently definite and thus withdrawal of the pending rejections under 35 USC § 112, second paragraph, are respectfully requested.

### Double Patenting

Claim 87 stands objected to under 37 CFR 1.75 as being a substantial duplicate of claim 88. In light of the above amendment, canceling Claim 88, Applicants respectfully request withdrawal of this objection.

### 35 USC § 102(e)

Claims 51-58, 60-62, 64-73, 79, 80, 82, 83, 85-89 and 93 stand rejected under 35 USC § 102(e) as anticipated by Wohlstadter et al., US Patent No. 6,066,448 (“Wohlstadter”). Wohlstadter is a continuation-in-part of USSN 08/402,076, filed March 10, 1995, which is a continuation-in-part of USSN 08/402,277, also filed March 10, 1995. Accordingly, the earliest possible priority date available for the disclosure in Wohlstadter is March 10, 1995.

In response to the rejection, Applicants are submitting herein an unsigned Declaration under 37 C.F.R. §1.131 by the inventors, Thomas J. Meade and Jon F. Kayyem, referencing experiments reflected in laboratory notebooks dated prior to March 10, 1995. A signed copy of the declaration will be forwarded as soon as possible. The declaration outlines that the invention was completed in this country prior to March 10, 1995. Accordingly, Wohlstadter is not a prior art reference, and Applicants respectfully request withdrawal of the rejections under 35 USC § 102(e).

### **35 USC § 103(a)**

Claims 59, 63, 81, 84 and 85 stand rejected under 35 USC § 103(a) as being unpatentable over Wohlstadter in view of Kayyem et al., U.S. Patent No. 6,096,273 (Kayyem).

As discussed above, Wohlstader is not a prior art reference, and thus cannot be combined with Kayyem as described by the Examiner when making the instant rejections. Accordingly, Applicants respectfully request withdrawal the rejections under 35 USC 103(a).

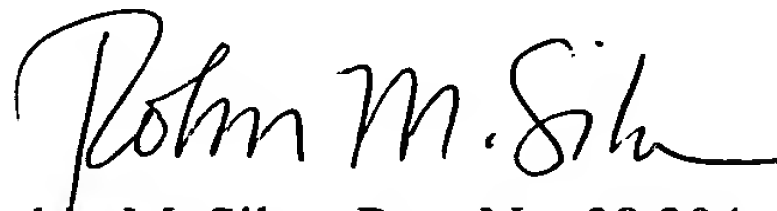
## CONCLUSION

Applicants respectfully submit that the claims are in condition for allowance and early notification to that effect is respectfully requested. Please direct any calls in connection with this application to the undersigned attorney at (415) 781-1989.

Respectfully submitted,  
DORSEY & WHITNEY LLP



4 Embarcadero Center,  
Suite 3400  
San Francisco, California  
94111-4187  
(415) 781-1989



Robin M. Silva, Reg. No. 38,304  
Filed under 37 CFR §1.34(a)



PATENT

Attorney Docket No. A-64411-2 (468267-00067)/RMS/RMK/SPL

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of

MEADE, *et al.*

Serial No.: 09/921,645

Filed: August 3, 2001

For: *Metallic Solid Supports Modified  
with Nucleic Acids*

Group No. 1637

Examiner: Strzelecka, Teresa, E.

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, Box 1450, Alexandria, VA 22313-1450 on:

Date:

Signature

Steve Lendaris

**DECLARATION PURSUANT TO 37 C.F.R. §1.131**

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313

Sir:

We, Thomas J. Meade and Jon F. Kayyem hereby declare as follows:

1. We are the inventors on the above-identified patent application and are familiar with its contents. We have also reviewed the pending claims in this application.
2. We are familiar with the Office Action mailed on November 17, 2003 wherein claims 51-69, 71, 72, and 74-93 were rejected over Wohlstadter et al. (6,066,448) which has an earliest possible priority date of March 10, 1995.
3. All of the ideas detailed in the above-identified application were contemplated in this country prior to March 10, 1995. This is evidenced by the appended documents.
4. One of the goals of the project that led to the filing of the parent application was to create a surface comprising a self-assembled monolayer with single stranded nucleic acids attached (referred herein as probes), and then to answer three questions: first, whether a solution-based complementary strand would bind to the probe; second, would a complementary strand attached to an atomic force

microscopy (AFM) tip bind to the probe, and if so; third, whether or not we could determine the force necessary to “tear apart” the duplex.

5. The experiments started out with the synthesis of the monolayer portion using an HO-(CH<sub>2</sub>)<sub>16</sub>-OH to form a molecule with a protected sulfur group (for attachment to a gold surface) on one end, to which a phosphoramidated nucleic acid was attached. The experiments proceeded with the coating of a gold surface with this monolayer-forming material. A photolithographic mask, with 8 x 8 micron squares on it, was then used to cover the gold surface. The surface was then exposed to a photoactivated agent and a mercury arc lamp which resulted in the ablation nucleic acids from the squares not covered by the mask. We then added a fluorescent complement to the surface, and viewed it under a confocal microscope. This resulted in a pattern of “light”, e.g. fluorescent, background, where the fluorescent solution based probes were found, and “dark” squares, where the surface-bound single stranded nucleic acid had been ablated off, and therefore no fluorescent probe was detected. A montage of several of these images, made over the course of the experiments, is attached as Exhibit A.
6. With regard to timing of these experiments, the documents attached as Exhibit B are pages from my notebook detailing the synthesis of some of the compounds used in these experiments. (Please note that all experiments not relevant to the present discussion have been redacted, as have all dates.) For example, page 136 documents the conversion of the HO-(CH<sub>2</sub>)<sub>16</sub>-OH molecule to the asymmetrical HO-(CH<sub>2</sub>)<sub>16</sub>-OAc needed for further reactions. The bottom of page 139 and the top of page 140 show the synthesis of the protected thiol-(CH<sub>2</sub>)<sub>16</sub>-OH molecule. the top of page 141 shows the reaction of the protected thiol-(CH<sub>2</sub>)<sub>16</sub>-OH molecule added to a phosphoramidite moiety. In conclusion, the invention was completed in this country prior to March 10, 1995.
7. We declare further that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such

willful statements may jeopardize the validity of the application or any patent  
issuing thereon.

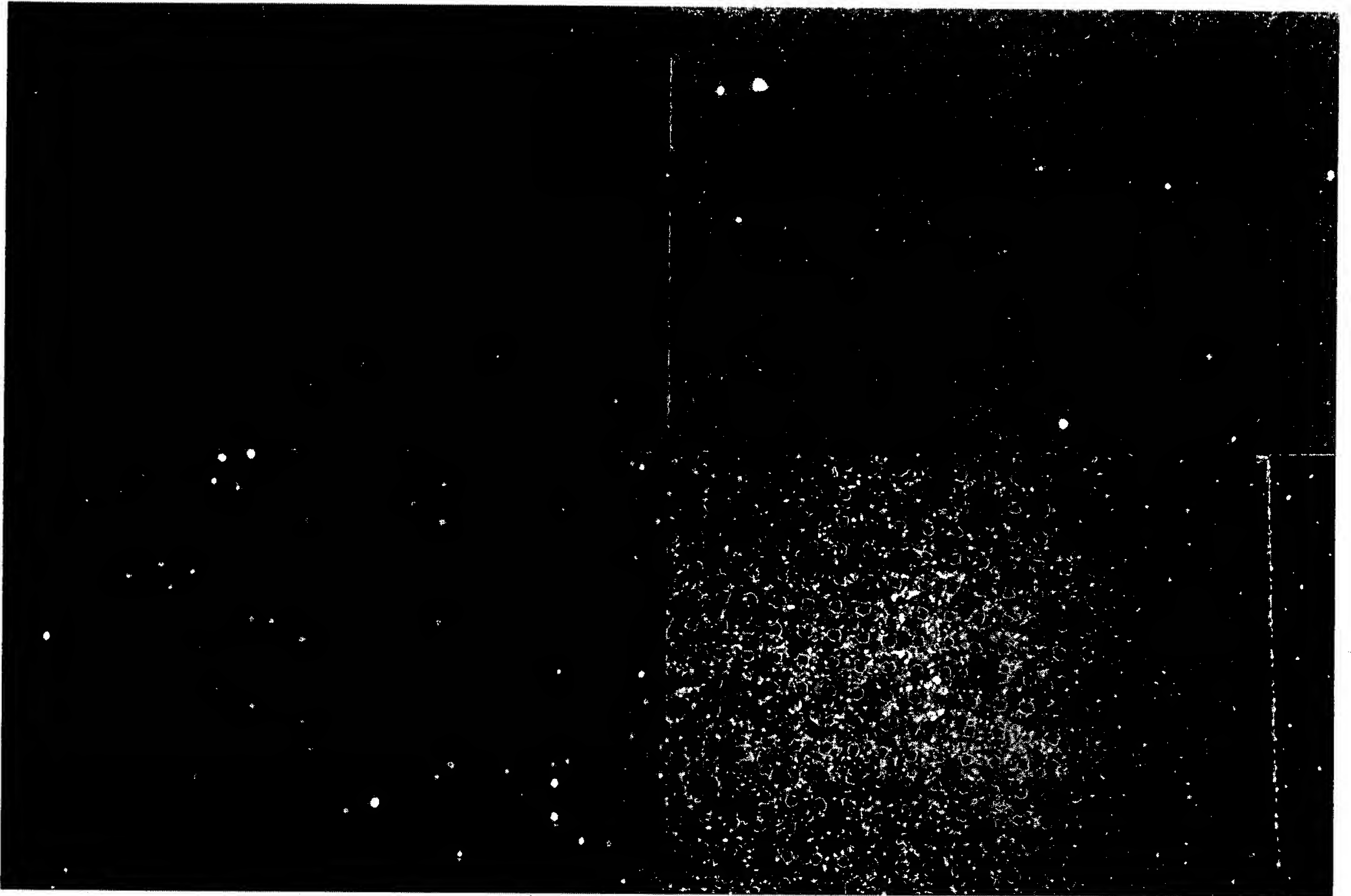
Date: \_\_\_\_\_  
Thomas J. Meade

Date: \_\_\_\_\_  
Jon F. Kayyem

UNSIGNED



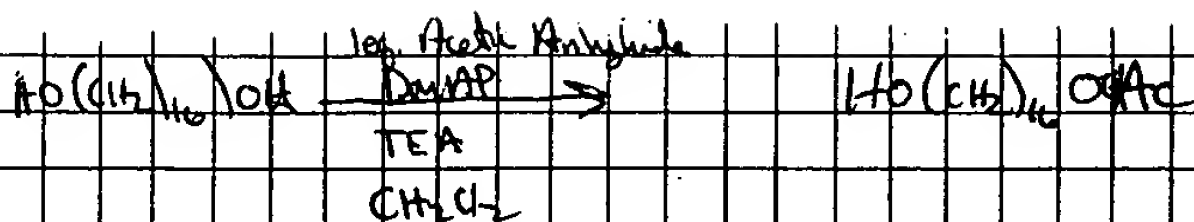
EXHIBIT A



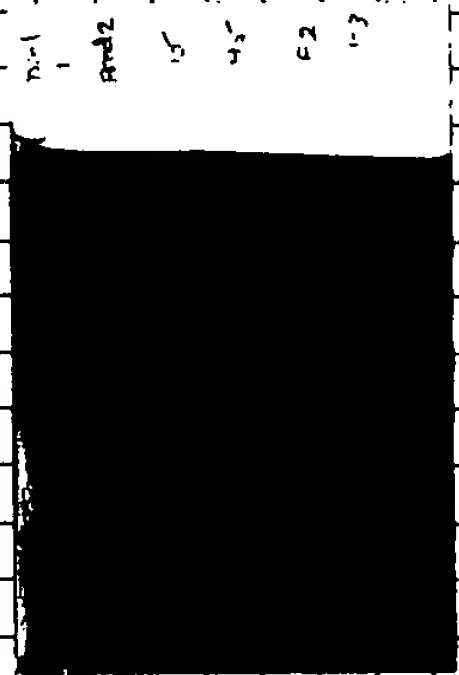




# EXHIBIT B



0.5 gr of  $\text{HO}(\text{CH}_2)_{10}\text{OH}$  (mw 258.45;  $1.9 \times 10^{-3}$  moles) was placed in a small round bottom and 20 mls of  $\text{CH}_2\text{Cl}_2$  added along with 0.05 equiv ( $9.7 \times 10^{-5}$  moles) (22.7 or 11.8 mgs) and 1.4 equiv of TEA (4.7 ml) and 1 equiv of Acetic Anhydride (mw 102.19; d = 1.08) [10.57 moles/liter] or 173 ml.



50/50 ether/hexane

Diol  
Std  
React

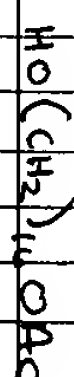
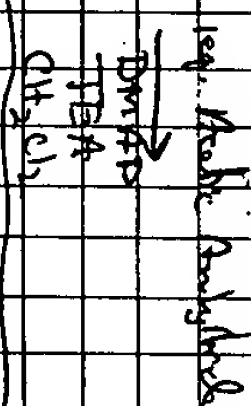
continuous extraction

50/50  
ether/hexane

Recr. 2

2.05 gr ( $7.93 \times 10^{-3}$  moles) was placed in a 100 ml RBF and 60 mls of  $\text{CH}_2\text{Cl}_2$  added along with 0.05 equiv DMAP (48 mg) and 1.4 equiv of TEA (17 mls) and 6.95 ml of Acetic Anhydride

## Reaction 3

3.00 g of  $\text{HO}(\text{CH}_2)_{10}\text{OH}$ 

70 mg DMAP

2.49 ml TEA

1.02 ml of Acetic Anhydride

100 ml RBF / 60 ml of  $\text{CH}_2\text{Cl}_2$ 

Duc. 4

1.45 g of  $\text{HO}(\text{CH}_2)_{10}\text{OH}$ 

23.4 mg DMAP

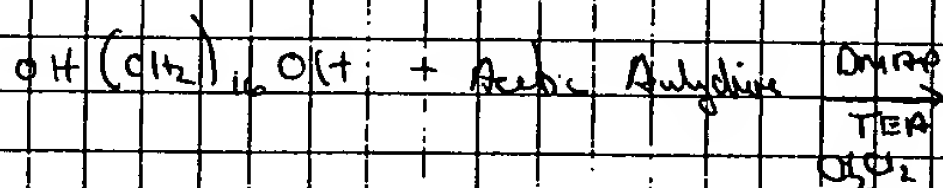
1.2 ml TEA

484 ml Acetic Anhydride

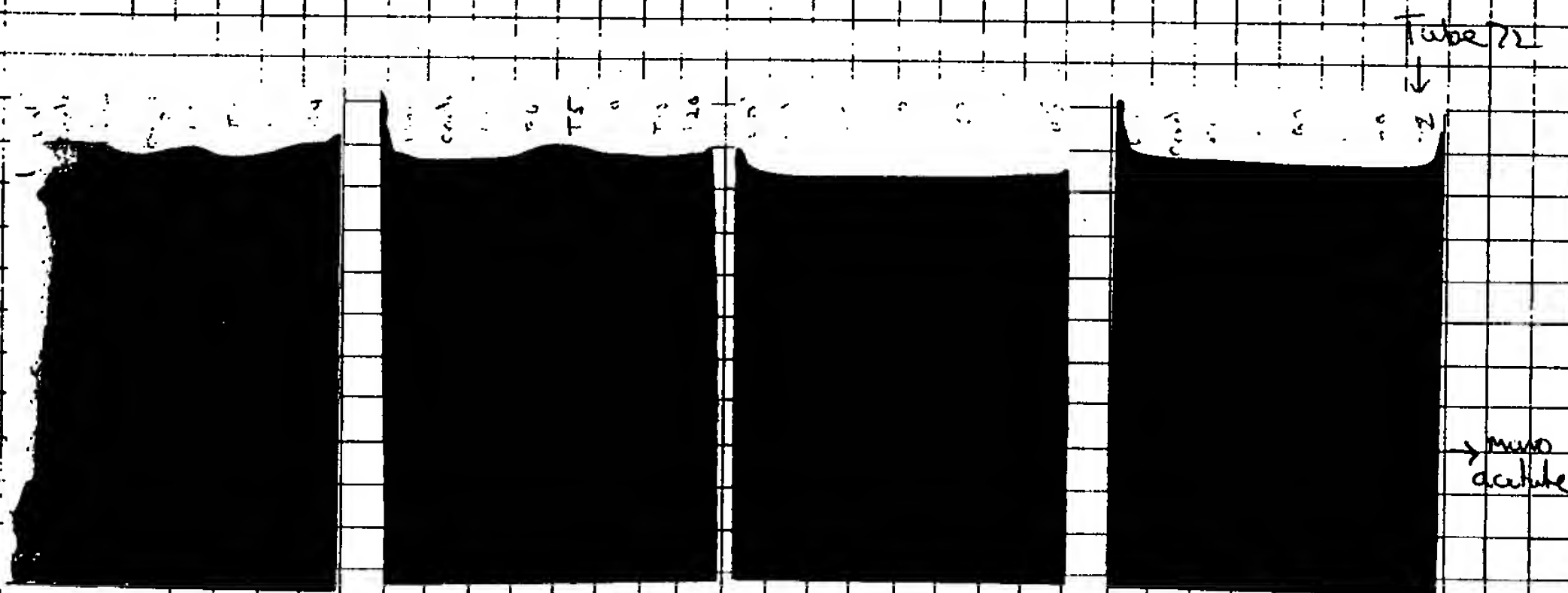
pure 5  
 98%  
 2.49 ml TEA  
 1.02 ml Acetic Anhydride  
 100 ml RBF  
 60 ml  $\text{CH}_2\text{Cl}_2$

138

Rec 3



1.37 gr of Diol was dissolved in 25ml of dry  $\text{CH}_2\text{Cl}_2$ . 0.5 equiv of DMAP (32.33 mg) was added and 1.4 equiv of TEA (1.14 ml) and 476  $\mu\text{l}$  of Acetic Anhydride.



50 ml solvent from 7 mm diol .05  
12 mm mono acetate = .25  
35 mm diacetate = Rf = 0.7

The flash column was

20 250ml of silica

1.2 liters of 180/20 hex/Ether  
was poured through and the  
gradient pushed up to 50

55, 45, After tube 72

it took and 200ml of 50/50

to remove all the product



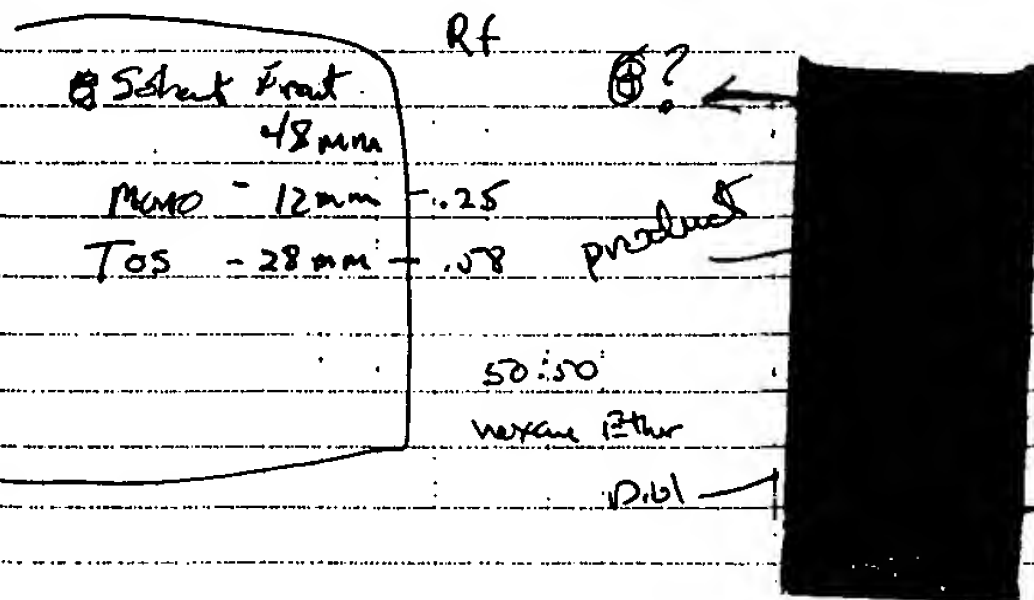
→ ?  
→ mono acetate  
→ Diol 50/50 Ether/hexane

$\text{C}_{18}\text{H}_{36}\text{O}_4$   
316  
300.93



Fisher & Porter page 1180

500mg ( $1.7 \times 10^{-3}$  moles) of  $\text{HO}(\text{CH}_2)_{16}\text{O}^{\text{O}}\text{CCH}_3$  in in dry pyridine  
 and in 50 ml solvent flask and cooled to  $0^\circ\text{C}$ . & TsCl  
 (90.65) in 1 molar excess (or 634 mgs) with a string bar  
 and allowed to proceed for 40 hrs. The solution was poured  
 into a beaker with 200mls of ice water and solution filtered  
 and then ~~solvent~~ <sup>shaken</sup> in heptane (20) and returned to

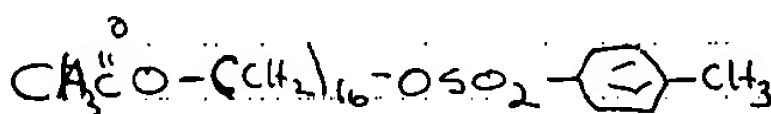
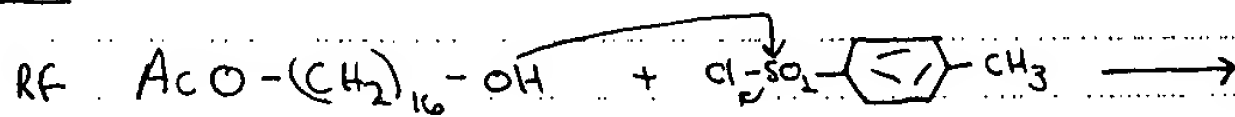


The product spot is UV active  
 and <sup>19</sup>F active  
 presumably the  $\text{H}_2\text{SO}_4$  in the  
 CAM hydrolysis the  $\text{H}_2\text{O}$   
 on Tos  $\text{H}_2\text{O}$  giving the  
 (+)  $\text{H}_2\text{SO}_4$

diagram.  $^1\text{H-NMR}$  is consistent with the proposed structure

Repeat

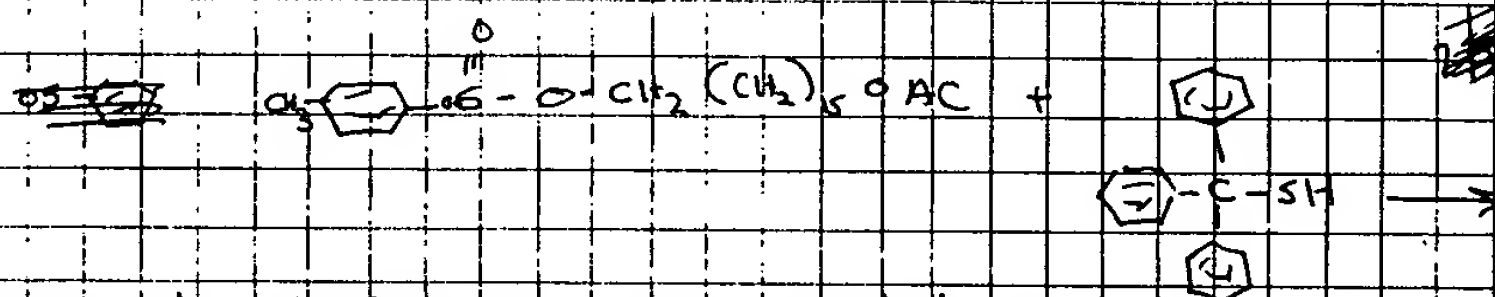
Solvent  
 Front = 47  
 Tos 21.5  
 Rf: 0.59



Total mass = 17.36 MW

$$\begin{aligned} \text{C}_{25}\text{H}_{42}\text{O}_5 &= 454.72 \\ \text{C}_{25}\text{H}_{42}\text{O}_5 &= 438.72 \\ &+ 15.998 \end{aligned}$$



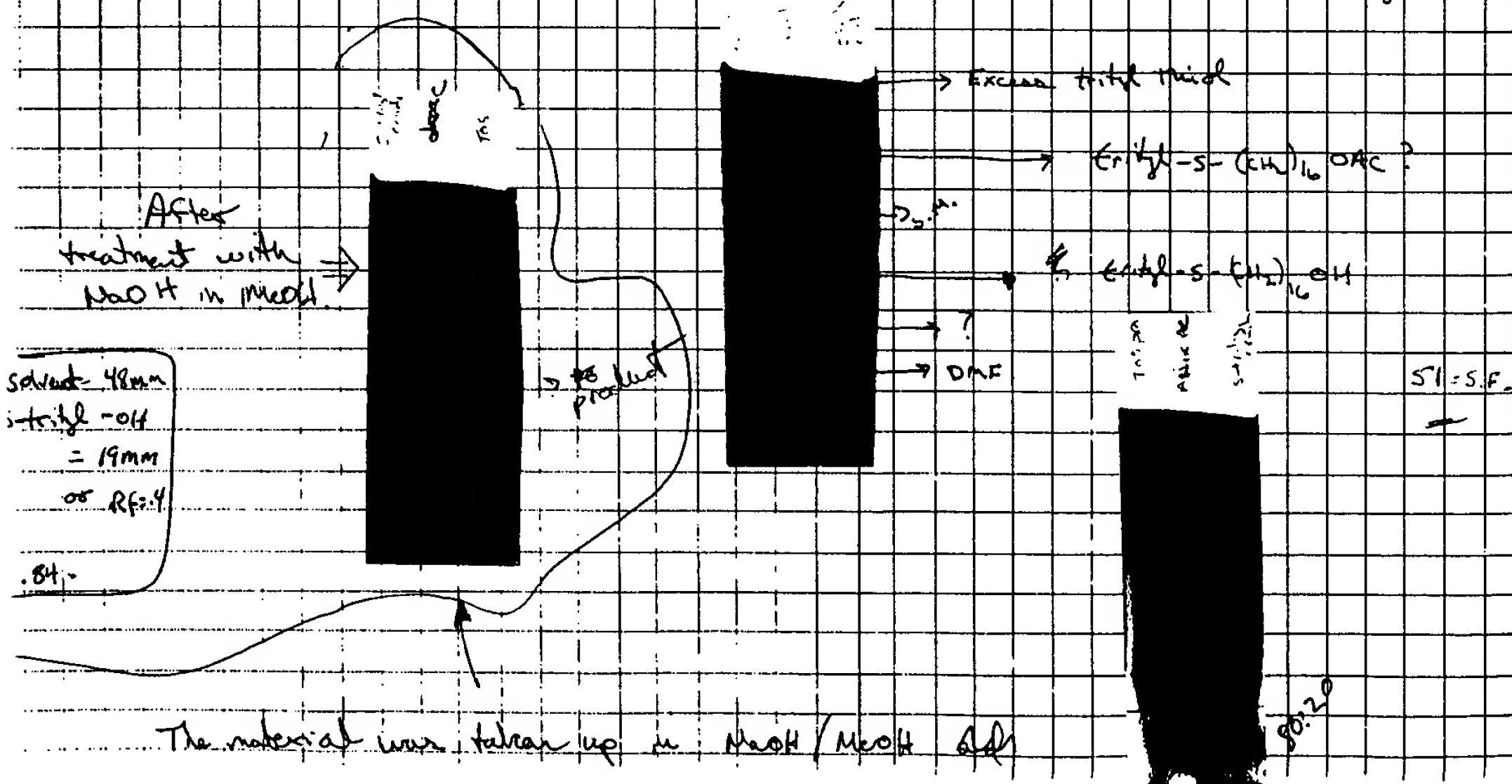


370 mg of  $\text{Tso}(\text{CH}_2)_{16}\text{OAc}$  was dissolved in  
 10 ml of DMF, and thoroughly degassed on the vac. line  
 ( $n_{\text{D}} = 1.45472$  or  $8.1 \times 10^{-4}$  moles)

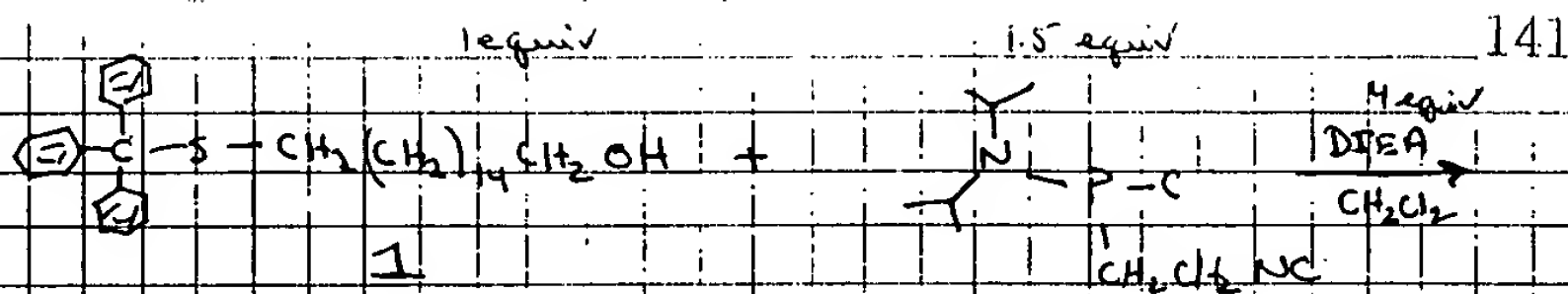
NaOH - 40

- Triphenyl methyl mercaptane ( $n_{\text{D}} 276.60$ , 0.95 equiv =  $7.7 \times 10^{-4}$  moles  
= 213 mg)
- 31.8 mg of NaOH (.98 equiv) in more of  $\text{H}_2\text{O}$   
180  $\mu\text{l}$

5 ml of ethanol was degassed on the vac line and 213 mg of  
 triphenyl added. @ 180  $\mu\text{l}$  of degassed NaOH in  $\text{H}_2\text{O}$  was added  
 via syringe under  $\text{Ar}$ . To this solution 370 mg of  
 $\text{Tso}(\text{CH}_2)_{16}\text{OAc}$  in DMF/EtOH was added and the solution degassed.







- 220 mg of **1** =  $4.4 \times 10^{-4}$  moles
- $6.5 \times 10^{-4}$  moles of phosphoramidite 236.68  $d = 1.061$   $M = 4.48$  ml
- 5.74 ml of ~~2.0 x 10~~  $1.8 \times 10^{-3}$  moles DIEA MW ~~101.99~~ 129.25  $\frac{0.742}{129.25} = 5.74 \text{ ml}$

220 mg of **1** was slurried in 15 ml of dry  $\text{CH}_2\text{Cl}_2$  and immediately 326  $\mu\text{l}$  of DIEA was added. 145  $\mu\text{l}$  of *O*-acetyl phosphoramidite was added dropwise. After 30 min and additional 50  $\mu\text{l}$  of *O*-acetyl reagent was added.

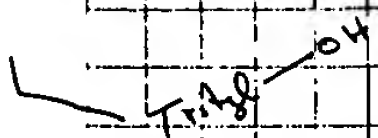
Note:

TEA MUST be present during flash!

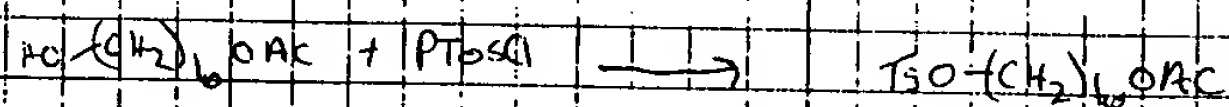
$\therefore$  at the addition of 1%

1% TEA to the mobile

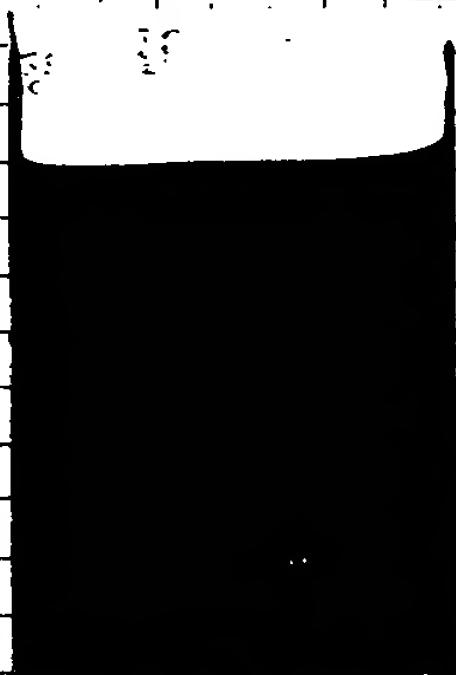
phase, drop the diethyl ether



142



51 gr of monoac product was stirred in 35 ml of dry pyridine and cooled to 0°C. 650 mg of TsCl was added and the reaction mixture allowed to proceed for 40 hrs.



→ monoac

Note:

Must use FRESH TsCl

Total yield 150 mg

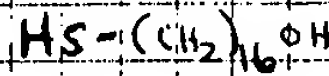
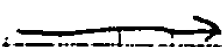
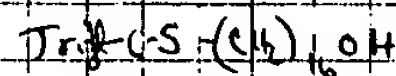
50/50 Ethyl/Hexane



(576.89)

270.54

143



~~20 mg~~ 11 mg of  $\text{Trityl-S-(CH}_2)_6\text{OH}$  ( $2.13 \times 10^{-5}$  moles)

276.40

$\begin{array}{r} - 1.01 \\ \hline 275.39 \end{array}$

+

576.89

0.3 mmol

100 mM TEAE

100 mM  $\text{AgNO}_3$

140 mM DTT

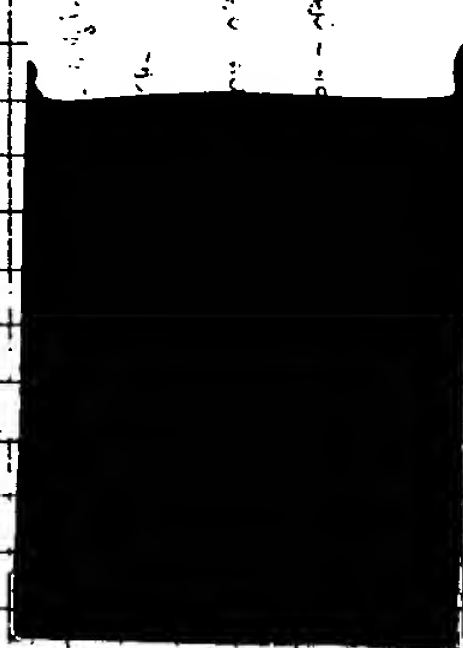
1 mg dissolved in MeOH (5 ml) and adding TEAE buffer

1 ml of  $\text{AgNO}_3$  solution - 30 min

1 ml of DTT + 30 min ( $154.2 \text{ mM}$ )  $\cdot 152.5 \text{ mM}$   
= 1 M

1.1 ml of 100 mM DTT  
1.1 ml of 100 mM  $\text{AgNO}_3$

or 1.1 ml of 1 M DTT  
or 1.1 ml of 1 M  $\text{AgNO}_3$



NMR says NR. The majority of isolated material is strongly nitrated.



144



+ NaSH Dry DMF  
 1.5 equiv  
 2 equiv

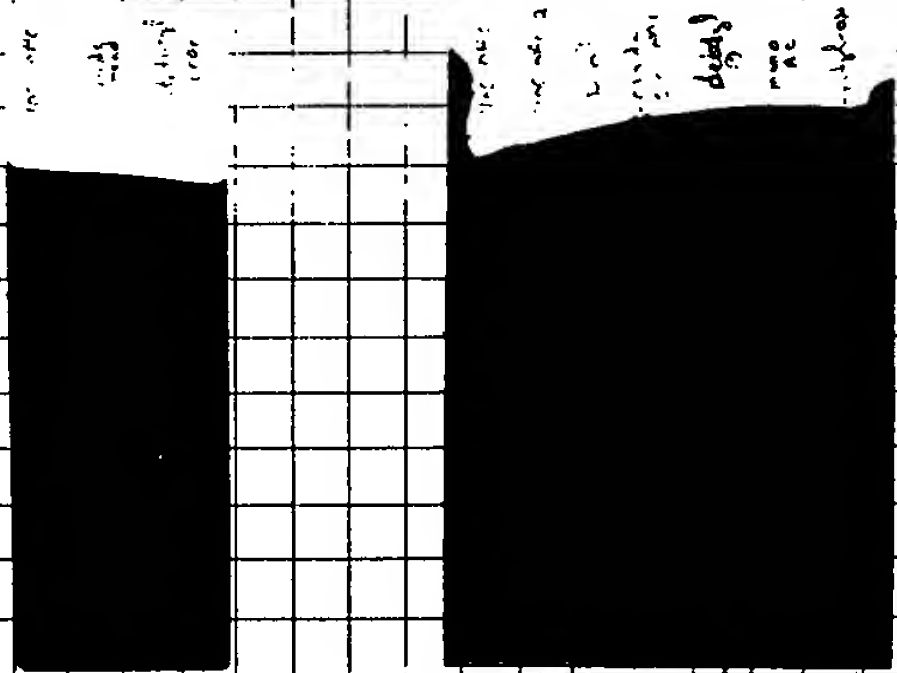
O.

MW = 454.72

or  $3.3 \times 10^{-4}$   
 or 18.4  $\mu\text{g}$ .

NaI + Anhydrous Acetone

Eryth + AgNO<sub>3</sub>  
 reaction



NMR reveals that the  
 spot detected ① is from  
 the reaction of Eryth-OH  
 with AgNO<sub>3</sub> did not occur

NMR = Eryth-OH NR

50:50  
 dichl  
 ether: Hexane

N.R.

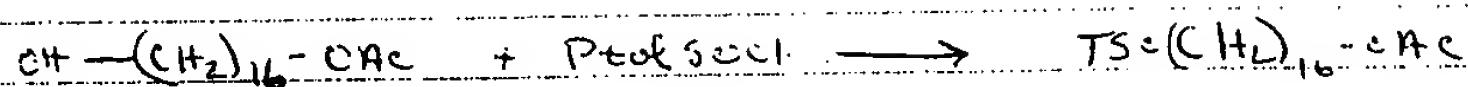
$\approx 75 \text{ mgs}$  starting material =  $1.7 \times 10^{-4}$  moles  $\times 1.2$  equiv of NaOH  
 $2 \times 10^{-4}$  moles NaOH or 8 mgs dissolved in MeOH



Aldose  
 NaOH

50:50  
 dichl  
 ether

with "Anhydrous" NaSH



$2 \times 250\text{mg}$  or  $8.5 \times 10^{-4}\text{ moles}$  in 15 mls of dry pyridine is

cooled to  $0^{\circ}\text{C}$  in an ice bath 320 mgs of  $\text{TsOCl}$  is added

and the reaction allowed to proceed ~~in~~ @  $4^{\circ}\text{C}$ . The product

(brownish tint) is poured into a beaker with woods of

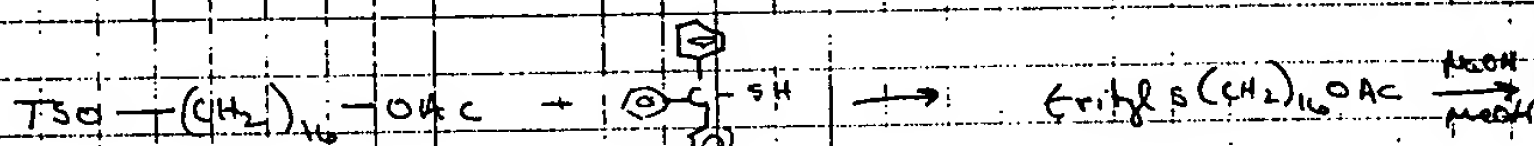
ice water, stirred for 10 min and filtered. The solid is

washed with water and dissolved in pet ether and

Charcoal added with stirring. The mixture was filtered

and dried (young total)

2.4 hours is Not long enough, some other  
remedy



325 nps ( $m = 454.72$ ) or  $7.115 \times 10^{-4}$  moles was dissolved in 10 ml of

dry DMF and degassed. Triphenyl methyl mercaptan (new 276.4) with

1.1 equiv ( $7.86 \times 10^{-4}$ ) or 217 mgs. ~~and~~ 1.05 equiv of NaOH or 30 mgs

was dissolved in 150 ml of  $H_2O$  and degassed.

The triethyl SH was dissolved in 5 ml of EtOH and degassed. The NaOH

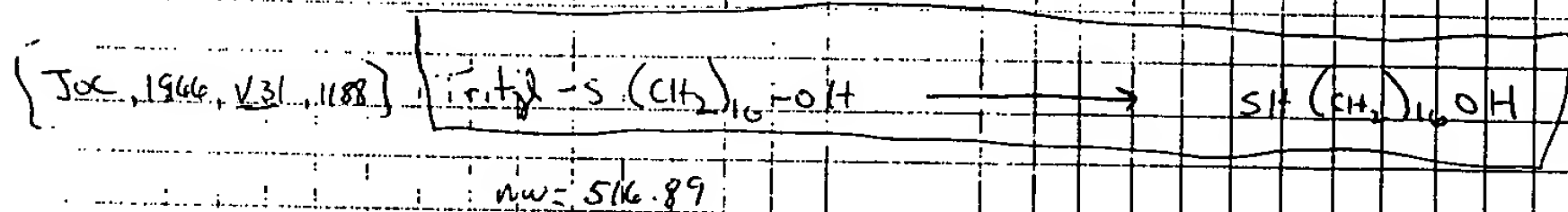
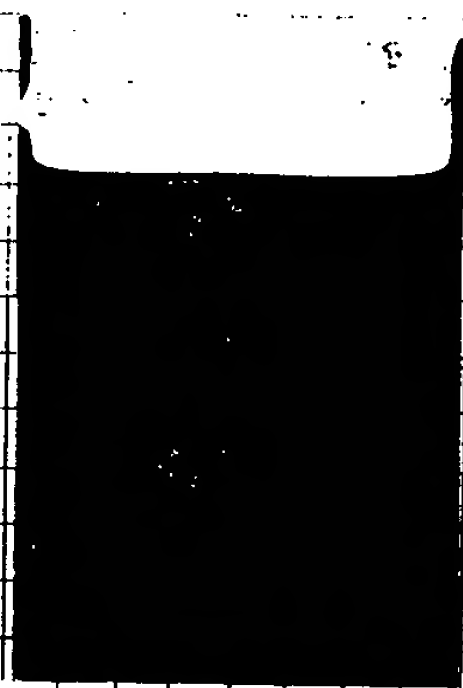
was added via syringe, and then <sup>to the</sup> TSO - otc, ~~added~~. The reaction

was repeatedly degassed and allowed to precool for 12 hrs.

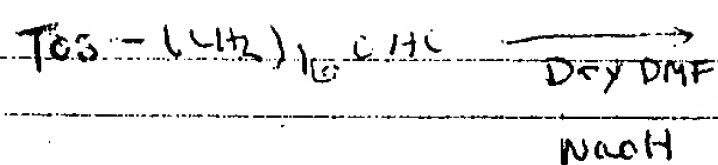
The reaction was:  $\text{THC}_2$  and  $\text{NaOH}/\text{MeOH}$  added to deacetylate

⇒ Flash  $\rightarrow$  phosphoric acid

EtOAc



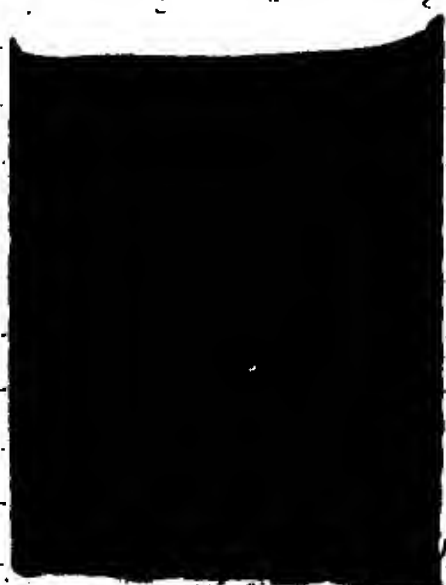
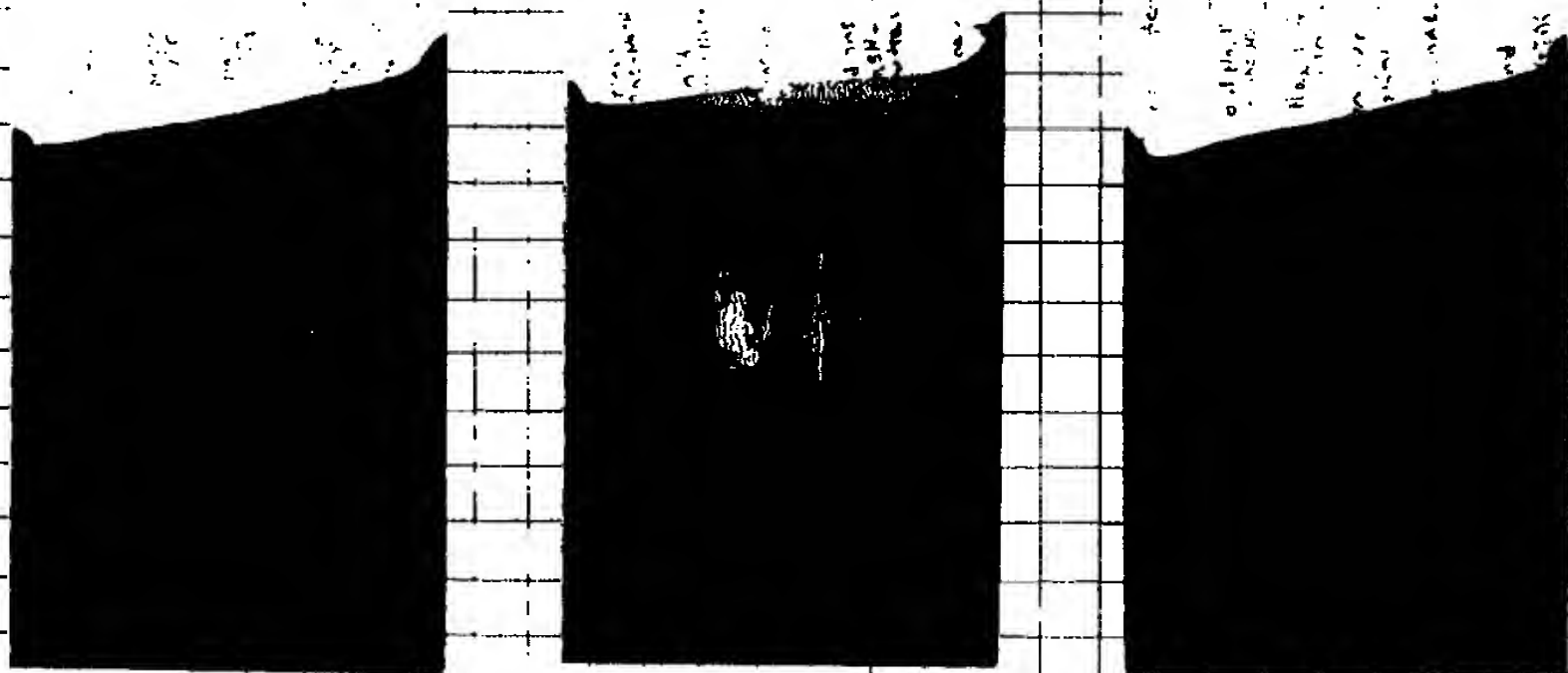
25 mg ( $4.8 \times 10^{-5}$  moles) was dissolved in <sup>glacial</sup> acetic acid (1.15 ml) and .38 ml of 1N HCl added. The reaction was allowed to stir for 1.5 hrs and 75°C. Upon addition of 1 ml, the material formed a white ppt immediately. After warming for ~ 5 min the ppt redissolved with the resulting soln slightly cloudy.



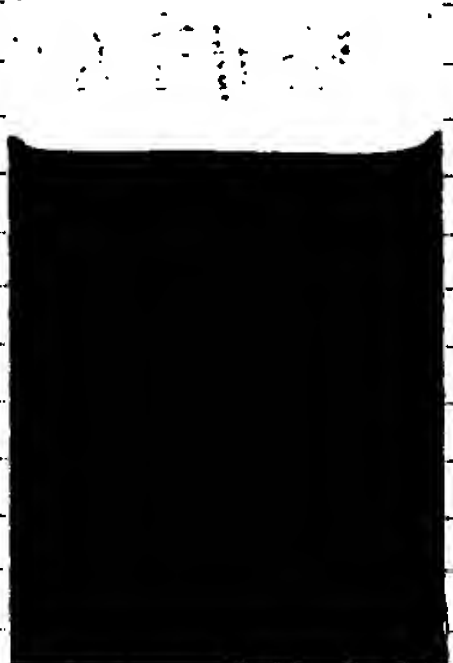
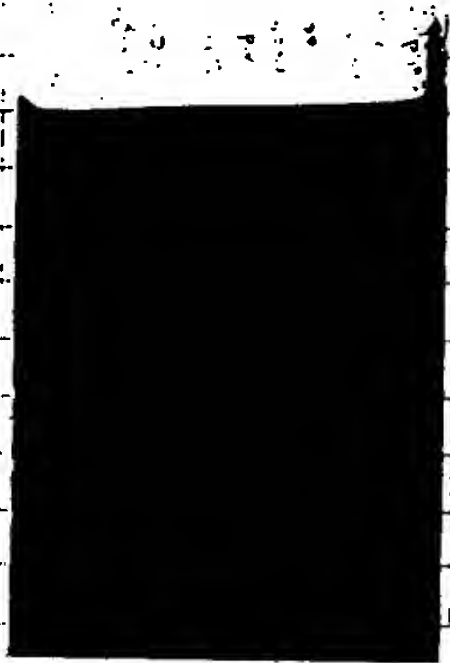
MW = 457.72

6.5 mg of the Tos derivative was dissolved in 5 ml of dry DMF.  
 $1.4 \times 10^{-4}$  moles  $\times$  2 equiv of NaSH or  $2.9 \times 10^{-4}$  moles or  $\frac{17}{16}$  mg

mg of NaOH is dissolved in 1 ml of dry MeOH. The NaSH  
 (in 2 ml of dry MeOH) was degassed and the NaOH added.  
 This solution is added (via syringe) to the dry DMF Tos  
 product.

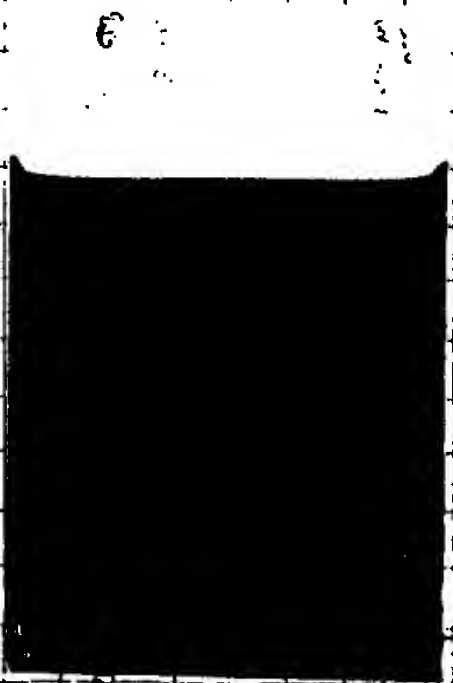






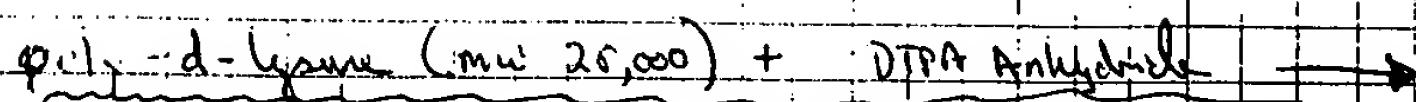
- A plate using ~~Ammonium~~ <sup>Ammonium</sup> Calcium Nitrate revealed on the presence of DTT.

After cooling a white ppt. formed and the solution was dissolved in 100 mL of  $\text{CH}_2\text{Cl}_2$ . pH  $\text{NaCO}_3$  (5M, pH 9) was used to wash the  $\text{CH}_2\text{Cl}_2$ . The reaction was conducted until the water layer was pH 8. The solution was then washed with pH 7 buffer, returned to dryness.



→ Before base wash  
↳ After washing (neutralized) with pH 8  $\text{NaCO}_3$





Polylysine was dissolved in  $\text{D}_2\text{O}$  and (20mg in 2mls) and applied to a PD-10. An additional  $\frac{1}{2}$  ml was added and 3 mls total collected from each of 3 PD-10 columns. The recovered dried material ( $\approx 6.5$  mgs) was divided into 3 reaction vessels of 22 mgs each.

The following ratios will be prepared

50x  
100x  
200x

(See page 100 + 101)

0.1 mM poly-d-lysine:

22 mgs ( $8.6 \times 10^{-7}$  moles) in  $\boxed{8.6 \text{ mls}} = 0.1 \text{ mM}$

$\therefore$

50x DTPA Anhydride or  $4.3 \times 10^{-5} \times 357.3 = 15.4 \text{ mgs}$

100x or  $8.6 \times 10^{-5} \times 357.3 = 30.7 \text{ mgs}$

200x or  $1.72 \times 10^{-4} \times 357.3 = 61.5 \text{ mgs}$

0.5 M  $\text{H}_2\text{CO}_3$  buffer; pH  $\approx$  9.5

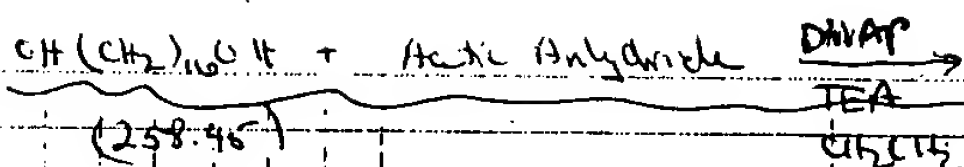
Only the 200x reaction showed any change in pH (e.g.  $\rightarrow$  9.0).

The reaction was ~~not~~ speed record to dryness

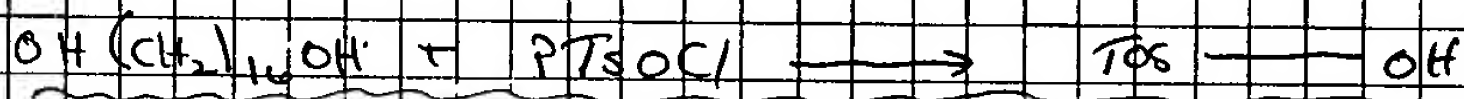
400x

35.2 mgs or  $1.4 \times 10^{-6}$  moles

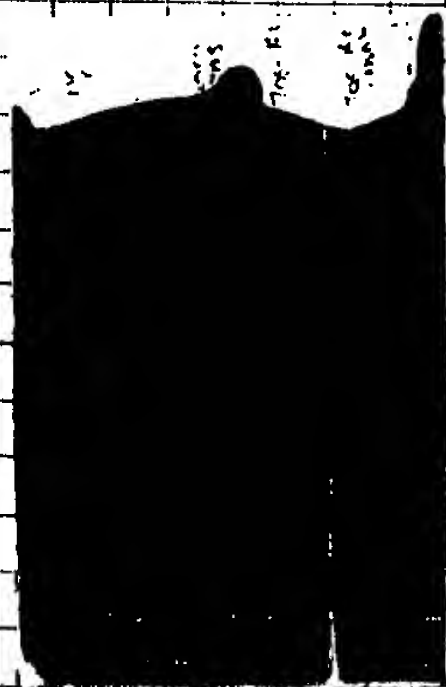
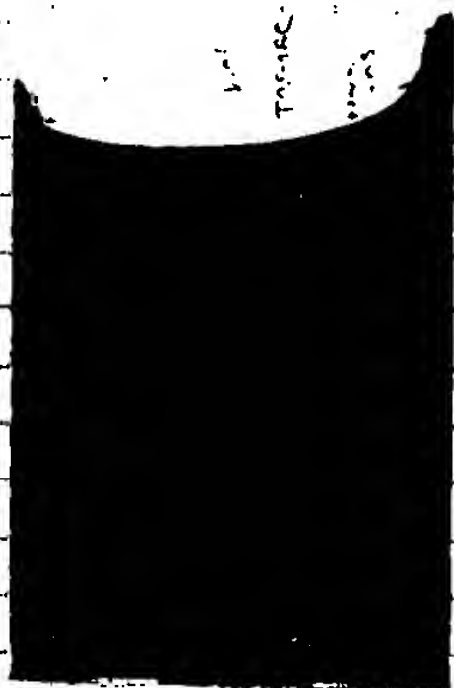
200 mgs of DTPA Anhydride in 14 mls pH 9.5  $\text{NaCO}_3$  Buffer



3 gr of diol was stirred in 50mls of dry  $\text{CH}_2\text{Cl}_2$  and  
~~70~~ 70 mg of DMAP added along with 2.5 mls of TEA  
 and 1.04 mls of Acetic Anhydride

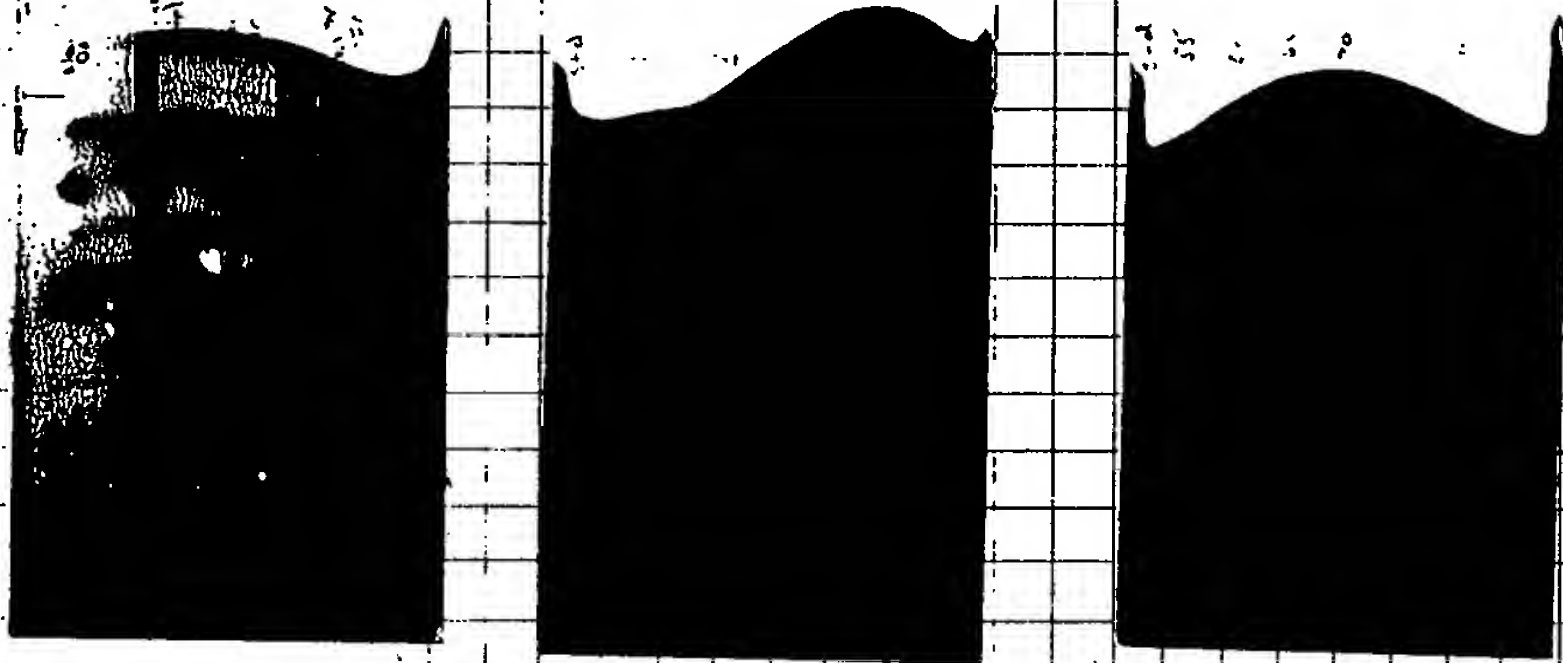


2 gr of  $(\text{OH(CH}_2\text{)}_{10}\text{OH}; 7.74 \times 10^{-3} \text{ moles})$  was <sup>stirred</sup> dissolved in 50 mls  
 of dry pyridine and cooled to  $0^\circ\text{C}$ . TsCl (190.65) equiv  
 = 1.48 grams was added and the reaction allowed to proceed  
 for





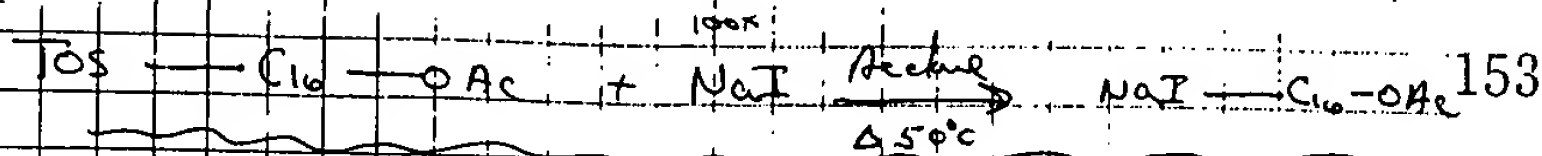
TOS—C16—OAc Purification



The water/py solution is expected to degum and skinned in  
heptane/diethyl ether and evaporated 3 times. The solid is  
dissolved in 50/50 ether:hexane and filtered. The solution is  
applied to a column 95:5 hexane:ether. Plate 2 reveals  
impurity. Future: Run 90:10 to start and move up to 80:20

See page 139 for prep.

~~139~~ 1



180 mg

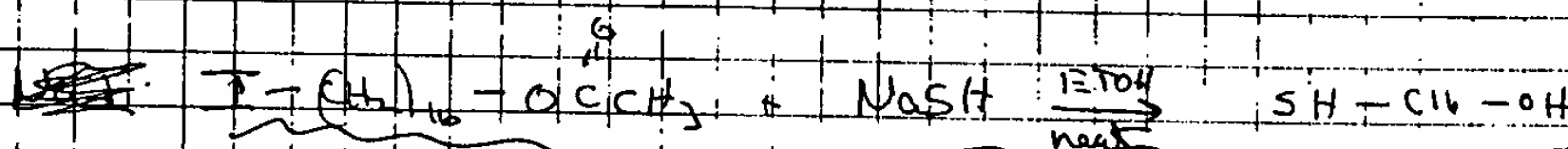
150

150

227 mg of  $\text{Tos} - \text{OAc}$  (mw = 454.72) or  $5.2 \times 10^{-4}$  moles is dissolved in

anhydrous Acetone and a  $100 \times$  or  $4.0 \times 10^{-2}$  moles of  $\text{NaI}$  (mw 149.89)

of  $\text{NaI}$  added. The rxn is followed by TLC. After 15 min a crystalline material began to ppt out.



50 mg of  $\text{I}(\text{CH}_2)_{16}\text{OAc}$  was dissolved in ~ 5 mL of EtOH.

75 mg of  $\text{NaSH}$  (10x) was

slurried in 2 mL of EtOH

and the iodide added slant.

and kept at ~ 60°C for 2 hrs.